DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

Project Information

1. **Proposal Title:**

   DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

2. **Proposal applicants:**

   ERICH FISCHER, Environmental Science Associates
   LARRY RIGGS, BIOSPHERE GENETICS
   PHIL LEITNER, ST. MARY'S COLLEGE
   FRANCIS VILLABLANCA, CALIFORNIA POLYTECHNIC UNIVERSITY, SAN LUIS OBISPO
   THOMAS LEEMAN, ENVIRONMENTAL SCIENCE ASSOCIATES
   NIALL McCARTEN, ENVIRONMENTAL SCIENCE ASSOCIATES
   CHRIS ROGERS, ENVIRONMENTAL SCIENCE ASSOCIATES

3. **Corresponding Contact Person:**

   ERICH FISCHER
   ENVIRONMENTAL SCIENCE ASSOCIATES
   700 UNIVERSITY AVENUE, SUITE 130 SACRAMENTO, CA 95825
   916 564-4500
   efischer@esassoc.com

4. **Project Keywords:**

   At-risk species, mammals
   Endangered Species
   Modeling

5. **Type of project:**

   Research

6. **Does the project involve land acquisition, either in fee or through a conservation easement?**

   No

7. **Topic Area**:

At-Risk Species Assessments

8. **Type of applicant:**

   Private for profit

9. **Location - GIS coordinates:**

Latitude:	38.186
Longitude:	-122.558
Datum:	NAD27

   Describe project location using information such as water bodies, river miles, road intersections, landmarks, and size in acres.

   PETALUMA MARSH WILDLIFE AREA, AN APPROXIMATELY 5,500-ACRE STUDY AREA.

10. **Location - Ecozone:**

    2.4 Petaluma River, 2.5 San Pablo Bay

11. **Location - County:**

    Sonoma

12. **Location - City:**

    Does your project fall within a city jurisdiction?

    No

13. **Location - Tribal Lands:**

    Does your project fall on or adjacent to tribal lands?

    No

14. **Location - Congressional District:**

    6th

15. **Location:**

    California State Senate District Number: 3

    California Assembly District Number: 6

16. **How many years of funding are you requesting?**

3 years

17. **Requested Funds:**
    a) Are your overhead rates different depending on whether funds are state or federal?

    No

    If no, list single overhead rate and total requested funds:

    Single Overhead Rate: 0%

    Total Requested Funds: $820,726

    b) Do you have cost share partners already identified?

    No

    c) Do you have potential cost share partners?

    No

    d) Are you specifically seeking non-federal cost share funds through this solicitation?

    No

    If the total non-federal cost share funds requested above does not match the total state funds requested in 17a, please explain the difference:

18. **Is this proposal for next-phase funding of an ongoing project funded by CALFED?**

    No

    Have you previously received funding from CALFED for other projects not listed above?

    No

19. **Is this proposal for next-phase funding of an ongoing project funded by CVPIA?**

    No

    Have you previously received funding from CVPIA for other projects not listed above?

    No

20. **Is this proposal for next-phase funding of an ongoing project funded by an entity other than CALFED or CVPIA?**

    No

    Please list suggested reviewers for your proposal. (optional)

HOWARD SHELLHAMMER	**SAN JOSE STATE UNIVERSITY (RETIRED)**	**408/258-9552**	**hreithro@pacbell.net**

21. **Comments:**

Environmental Compliance Checklist

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

1. **CEQA or NEPA Compliance**
   a) Will this project require compliance with CEQA?

      No
   b) Will this project require compliance with NEPA?

      No
   c) If neither CEQA or NEPA compliance is required, please explain why compliance is not required for the actions in this proposal.

      THIS IS A RESEARCH PROJECT WITH RESTORATION IMPLICATIONS, BUT NO RESTORATION AS PART OF THE PROJECT. THE ACTIVITIES PROPOSED DO NOT MEET THE DEFINITION OF "PROJECT" UNDER CEQA OR NEPA.

2. **If the project will require CEQA and/or NEPA compliance, identify the lead agency(ies).** *If not applicable, put "None".*

   CEQA Lead Agency:
   NEPA Lead Agency (or co-lead:)
   NEPA Co-Lead Agency (if applicable):

3. **Please check which type of CEQA/NEPA documentation is anticipated.**

   CEQA
   -Categorical Exemption
   -Negative Declaration or Mitigated Negative Declaration
   -EIR
   Xnone

   NEPA
   -Categorical Exclusion
   -Environmental Assessment/FONSI
   -EIS
   Xnone

   If you anticipate relying on either the Categorical Exemption or Categorical Exclusion for this project, please specifically identify the exemption and/or exclusion that you believe covers this project.

4. **CEQA/NEPA Process**
   a) Is the CEQA/NEPA process complete?

      Not Applicable

b) If the CEQA/NEPA document has been completed, please list document name(s):

5. **Environmental Permitting and Approvals** (*If a permit is not required, leave both Required? and Obtained? check boxes blank.*)

LOCAL PERMITS AND APPROVALS

Conditional use permit

Variance

Subdivision Map Act

Grading Permit

General Plan Amendment

Specific Plan Approval

Rezone

Williamson Act Contract Cancellation

Other

STATE PERMITS AND APPROVALS

Scientific Collecting Permit Obtained

CESA Compliance: 2081

CESA Compliance: NCCP

1601/03

CWA 401 certification

Coastal Development Permit

Reclamation Board Approval

Notification of DPC or BCDC

Other

FEDERAL PERMITS AND APPROVALS

ESA Compliance Section 7 Consultation

ESA Compliance Section 10 Permit Obtained

Rivers and Harbors Act

CWA 404

Other

PERMISSION TO ACCESS PROPERTY

Permission to access city, county or other local agency land.
Agency Name:

Permission to access state land.
Agency Name: CA DEPARTMENT OF FISH AND GAME — Required

Permission to access federal land.
Agency Name:

Permission to access private land.
Landowner Name:

6. **Comments.**

Land Use Checklist

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

1. **Does the project involve land acquisition, either in fee or through a conservation easement?**

   No

2. **Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?**

   Yes

3. **Do the actions in the proposal involve physical changes in the land use?**

   No

   If you answered no to #3, explain what type of actions are involved in the proposal (i.e., research only, planning only).

   THIS IS A RESEARCH PROJECT ONLY.

4. **Comments.**

Conflict of Interest Checklist

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

Please list below the full names and organizations of all individuals in the following categories:

- Applicants listed in the proposal who wrote the proposal, will be performing the tasks listed in the proposal or who will benefit financially if the proposal is funded.
- Subcontractors listed in the proposal who will perform some tasks listed in the proposal and will benefit financially if the proposal is funded.
- Individuals not listed in the proposal who helped with proposal development, for example by reviewing drafts, or by providing critical suggestions or ideas contained within the proposal.

The information provided on this form will be used to select appropriate and unbiased reviewers for your proposal.

Applicant(s):

ERICH FISCHER, Environmental Science Associates
LARRY RIGGS, BIOSPHERE GENETICS
PHIL LEITNER, ST. MARY'S COLLEGE
FRANCIS VILLABLANCA, CALIFORNIA POLYTECHNIC UNIVERSITY, SAN LUIS OBISPO
THOMAS LEEMAN, ENVIRONMENTAL SCIENCE ASSOCIATES
NIALL McCARTEN, ENVIRONMENTAL SCIENCE ASSOCIATES
CHRIS ROGERS, ENVIRONMENTAL SCIENCE ASSOCIATES

Subcontractor(s):

Are specific subcontractors identified in this proposal? Yes

If yes, please list the name(s) and organization(s):

LARRY RIGGS	BIOSPHERE GENETICS
PHIL LEITNER	PRIVATE CONSULTANT

Helped with proposal development:

Are there persons who helped with proposal development?

Yes

If yes, please list the name(s) and organization(s):

FRANK WERNETTE CALIFORNIA DEPARTMENT OF FISH AND GAME

LAURIE BRIDEN CALIFORNIA DEPARTMENT OF FISH AND GAME

LAUREEN THOMSEN CALIFORNIA DEPARTMENT OF FISH AND GAME

PATTY FINFROCK CALIFORNIA DEPARTMENT OF WATER RESOURCES

JOHN GUSTAFSON CALIFORNIA DEPARTMENT OF FISH AND GAME

Comments:

Budget Summary

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

Please provide a detailed budget for each year of requested funds, indicating on the form whether the indirect costs are based on the Federal overhead rate, State overhead rate, or are independent of fund source.

Independent of Fund Source

Year 1												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary** ***(per year)***	**Benefits** ***(per year)***	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	DEVELOP HSI MODEL	880	76,800		400	12,000	65,000	21,000		175200.0		175200.00
2	POPULATION GENETICS	136	13,240		200	300	88,500			102240.0		102240.00
		1016	90040.00	0.00	600.00	12300.00	153500.00	21000.00	0.00	277440.00	0.00	277440.00

Year 2												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary** ***(per year)***	**Benefits** ***(per year)***	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
1	DEVELOP HSI MODEL	472	41,880		500	800	65,000			108180.0		108180.00
2	POPULATION GENETICS	120	12,200				93,726			105926.0		105926.00
3	DETERMINE SPATIAL REQUIREMENTS	608	52,520			1,200				53720.0		53720.00
		1200	106600.00	0.00	500.00	2000.00	158726.00	0.00	0.00	267826.00	0.00	267826.00

Year 3												
Task No.	**Task Description**	**Direct Labor Hours**	**Salary** ***(per year)***	**Benefits** ***(per year)***	**Travel**	**Supplies & Expendables**	**Services or Consultants**	**Equipment**	**Other Direct Costs**	**Total Direct Costs**	**Indirect Costs**	**Total Cost**
2	POPULATION GENETICS	80	8,400				82,360			90760.0		90760.00
3	DETERMINE SPATIAL REQUIREMENTS	740	64,500		300		65,000			129800.0		129800.00
4	EVALUATE RESTORATION PRIORITIES	580	52,100			2,800				54900.0		54900.00
		1400	125000.00	0.00	300.00	2800.00	147360.00	0.00	0.00	275460.00	0.00	275460.00

Grand Total=820726.00

Comments.
THE $21,000 EQUIPMENT EXPENSE IN THE FIRST YEAR IS THE COST OF ACQUIRING AERIAL PHOTOGRAPHY AND OTHER DIGITAL DATA.

Budget Justification

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

Direct Labor Hours. Provide estimated hours proposed for each individual.

ENVIRONMENTAL SCIENCE ASSOCIATES: NIALL McCARTEN: 460 CHRIS ROGERS: 240 ERICH FISCHER: 1,320 THOMAS LEEMAN: 560 ASSOCIATE: 880 ADMINISTRATIVE STAFF: 156

Salary. Provide estimated rate of compensation proposed for each individual.

ENVIRONMENTAL SCIENCE ASSOCIATES: NIALL McCARTEN: 52,900 CHRIS ROGERS: 24,000 ERICH FISCHER: 125,400 THOMAS LEEMAN: 47,600 ASSOCIATE: 61,600 ADMINISTRATIVE STAFF: 10,140

Benefits. Provide the overall benefit rate applicable to each category of employee proposed in the project.

NOT APPLICABLE (SALARY INCLUDES BENEFITS).

Travel. Provide purpose and estimate costs for all non-local travel.

TOTAL TRAVEL COST IS $1,400 FOR TRAVEL TO AND FROM PETALUMA MARSH FOR FIELD VISITS.

Supplies & Expendables. Indicate separately the amounts proposed for office, laboratory, computing, and field supplies.

LAB SUPPLIES FOR GENETIC ANALYSIS:: $50,086 FIELD SUPPLIES FOR SMHM: $22,200 OTHER FIELD SUPPLIES (VEGETATION SAMPLING): $10,300 PRINTING/PLOTTER SUPPLIES, SOFTWARE, OTHER OFFICE SUPPLIES: $8,200

Services or Consultants. Identify the specific tasks for which these services would be used. Estimate amount of time required and the hourly or daily rate.

ST. MARYS COLLEGE: PHIL LEITNER (SALT MARSH HARVEST MOUSE CONSULTANT) SURVEY WORK FOR TASKS 1 AND 3: HOURS REQUIRED: 720 HOURLY RATE: $240 (CUMULATIVE FOR AN ENTIRE SURVEY CREW) BIOSPHERE GENETICS INC.: LARRY RIGGS ANALYSIS OF SALT MARSH HARVEST MOUSE POPULATION GENETICS FOR TASK 2: HOURS REQUIRED: 1,650 HOURLY RATE: $130

Equipment. Identify non-expendable personal property having a useful life of more than one (1) year and an acquisition cost of more than $5,000 per unit. If fabrication of equipment is proposed, list parts and materials required for each, and show costs separately from the other items.

GEOGRAPHIC INFORMATION SYSTEMS DATA - INCLUDES ACQUIRING HIGH RESOLUTION DIGITAL AERIAL IMAGERY FOR SUISUN MARSH AND ASSOCIATED DATA SETS (TOPOGRAPHY, HYDROLOGY, ROADS, ETC.): $21,000

Project Management. Describe the specific costs associated with insuring accomplishment of a specific project, such as inspection of work in progress, validation of costs, report preparation, giving presentatons, reponse to project specific questions and necessary costs directly associated with specific project oversight.

TOTAL COST OVER THREE YEARS: $18,540

Other Direct Costs. Provide any other direct costs not already covered.

NONE.

Indirect Costs. Explain what is encompassed in the overhead rate (indirect costs). Overhead should include costs associated with general office requirements such as rent, phones, furniture, general office staff, etc., generally distributed by a predetermined percentage (or surcharge) of specific costs.

NONE.

Executive Summary

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

THIS PROJECT WILL DEVELOP A HABITAT SUITABILITY MODEL BASED ON HABITAT RELATIONSHIPS AND POPULATION GENETICS OF THE SALT MARSH HARVEST MOUSE (REITHRODONTOMYS RAVIVENTRIS) IN THE NORTH BAY REGION (INCLUDING SAN PABLO BAY AND SUISUN BAY). THE INTENT OF THIS MODEL IS TO ASSIST IN RESTORATION EFFORTS ACROSS THE NORTH BAY BY IDENTIFYING AREAS THAT ARE IMPORTANT FOR MAINTAINING GENETIC VARIATION. THE MODEL ALSO MAY BE USED TO TARGET AREAS WHERE SPECIES REINTRODUCTIONS WILL HAVE A HIGHER LIKELIHOOD FOR SUCCESS. BY MODELING HABITAT AND EXAMINING GENETIC VARIATION AT THIS SCALE, WE WILL TAKE THE FIRST STEP TOWARD DEVELOPING A REGIONAL MANAGEMENT PLAN FOR SALT MARSH HARVEST MOUSE HABITAT RESTORATION. CONSEQUENTLY, THIS STUDY WILL PAVE THE WAY FOR A COMPREHENSIVE RECOVERY EFFORT. TO ACHIEVE THIS GOAL, WE WILL EXAMINE SEVERAL FACTORS CONSIDERED SIGNIFICANT TO SPECIES CONSERVATION, INCLUDING GENETIC VARIATION, HABITAT RELATIONSHIPS, AND METAPOPULATION STRUCTURE. THE MODEL WILL BE DEVELOPED USING DATA COLLECTED IN PETALUMA MARSH AND WILL BE TESTED IN OTHER REGIONS OF THE NORTH BAY. GENETIC INFORMATION WILL BE COLLECTED FROM PETALUMA MARSH AND OTHER NORTH BAY LOCATIONS; A COMPARISON OF THIS DATA WITH VOUCHER SPECIMENS WILL YIELD INFORMATION ON HISTORICAL VS. CURRENT GENETIC VARIATION. THIS INFORMATION MAY BE USED TO INFER HABITAT DISTRIBUTION REQUIREMENTS THAT WILL BE INTEGRATED INTO THE MODEL. ONCE FINALIZED, LAND MANAGERS AND RESOURCE AGENCIES MAY USE THE MODEL TO TARGET RESTORATION EFFORTS SPATIALLY, THEREBY PROVIDING A FOUNDATION TO BUILD REGIONAL CONSERVATION AND RESTORATION MANAGEMENT PLANS.

Proposal

Environmental Science Associates

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

ERICH FISCHER, Environmental Science Associates
LARRY RIGGS, BIOSPHERE GENETICS
PHIL LEITNER, ST. MARY'S COLLEGE
FRANCIS VILLABLANCA, CALIFORNIA POLYTECHNIC UNIVERSITY, SAN LUIS OBISPO
THOMAS LEEMAN, ENVIRONMENTAL SCIENCE ASSOCIATES
NIALL McCARTEN, ENVIRONMENTAL SCIENCE ASSOCIATES
CHRIS ROGERS, ENVIRONMENTAL SCIENCE ASSOCIATES

DEVELOPMENT OF A POPULATION-BASED HABITAT SUITABILITY MODEL FOR SALT MARSH HARVEST MOUSE TO GUIDE RESTORATION EFFORTS IN THE NORTH BAY REGION

A. Project Description

This project will develop a habitat suitability model based on habitat relationships and population genetics of the salt-marsh harvest mouse (*Reithrodontomys raviventris*) (SMHM) in the North Bay region (including San Pablo Bay and Suisun Bay). The intent of this model is to assist in restoration efforts across the North Bay by identifying areas that are important for maintaining genetic variation. The model also may be used to target areas where species reintroductions will have a higher likelihood for success. By modeling habitat and examining genetic variation at this scale, we will take the first step towards developing a regional management plan for SMHM habitat restoration. Consequently, this study will pave the way for a comprehensive recovery effort.

To achieve this goal, we will examine several factors considered significant to species conservation, including genetic variation, habitat relationships, and metapopulation structure. The model will be developed using data collected in Petaluma Marsh and will be tested in other regions of the North Bay. Genetic information will be collected from Petaluma Marsh and other North Bay locations; a comparison of this data with voucher specimens will yield information on historical versus current genetic variation. This information may be used to infer habitat distribution requirements that will be integrated into the model. Once finalized, land managers and resource agencies may use the model to target restoration efforts spatially, thereby providing a foundation to build regional conservation and restoration management plans.

1. Problem

Background. The habitat relationship between SMHM and common pickleweed within tidal and diked marshes is well-documented (Shellhammer et al. 1982, Shellhammer 1982, Fisler 1965, Wondolleck et al. 1976, Geissel et al. 1988). It also has been shown that the species requires an upland transition zone within tidal areas to take refuge at high tides or when preferred habitat is otherwise unavailable (Geissel et al. 1988, Botti et al. 1986). While recovery for the species is ongoing, restoration efforts have been very localized, and a larger picture of existing and potential habitat for the North Bay region has yet to be defined. In addition, restoration projects often have goals of simply increasing the area of tidal marsh; specific habitat components, such as upland transition areas, polygon attributes (such as topology), or linkages to other areas are often either not considered or only considered at a rudimentary level. Without a greater understanding of how regional conservation and restoration projects may affect this species, well-meaning restoration projects may fail to address significant conservation issues (such as genetic diversity, upland refugia, or maintaining a viable matrix).

Goals and Hypotheses. We have identified two primary goals of the project: 1) identify habitat relationships and genetic thresholds for sustainable populations in the North Bay region, and 2) develop a Geographical Information System (GIS)-based habitat capability model that may be

used to guide restoration efforts in the region. We hypothesize that there are significant barriers to gene flow and that habitat fragmentation has made some populations less viable. We further hypothesize that significant habitat relationships may be modeled at the landscape level and that in doing so, potential barriers to gene flow may be identified through combining the results of the landscape modeling to genetic information from existing populations. Methods to test these hypotheses are provided in Section 3.

Study Area. We chose Petaluma Marsh (Figure 1) as our study area for several reasons. Although the marsh may not represent an area that has the highest density of SMHM, it does represent a tidal marsh that has undergone relatively little modification in recent years. Because of this, it may represent a fairly stable ecological system that, while not necessary providing optimal habitat for SMHM in every instance, does provide for a persistent population over time. Our goal to model suitable SMHM will be built on the same principles; namely that a large, relatively stable system is more desirable for management than a smaller system that is more suspect to stochastic events.

We also chose Petaluma Marsh because of its well-documented history of harboring the species over time. The historical record for SMHM in the marsh includes 72 records of *R. r. halicoetes* by collectors from 1908 through 1959. These historical samples, as well as present-day trap records by the California Department of Fish and Game (DFG) and other researchers, provide an excellent sequence of temporal data. This data will be critical to our analysis of genetic variation within the marsh.

2. Justification

A conceptual model that integrates adaptive management concepts (as outlined in Chapter 2 of the Draft Stage 1 Implementation Plan) is provided in Figure 2. While our study clearly falls in the category of research, it will generate data that will be useful in identifying habitat polygons that are a high priority for restoration. We chose to address the issue of species recovery and associated habitat restoration by using two tools: habitat modeling at the landscape scale, and analyzing genetic variation at multiple scales, both spatially and temporally.

The need to conserve and recover endangered species at the landscape level is well-recognized (Burkey 1989, Fahrig and Merriam 1985, Merriam and Lanoue 1990, Noss and Harris 1986, Shaffer 1990). Furthermore, the recovery plan for SMHM (USFWS 1984) identifies the Petaluma Marsh Wildlife Area, as well as several other refuges and parks in the region as important for species recovery. The recovery plan, as well as the CALFED Draft Stage 1 Implementation Plan, has identified the need to either: 1) collect more data on the habitat requirements of SMHM in tidal, brackish, and non-tidal (diked) marshes; or 2) model habitat capability. The habitat suitability index (HIS) model will directly address these issues for SMHM in the North Region, and more specifically, *R. r. halicoetes*.

Generally speaking, there are four basic objectives involved in rare species preservation and restoration efforts that can benefit from genetic considerations and data. They are:

1. Preserve or restore individual populations or metapopulation assemblages.

2. Preserve, restore, and/or maintain genetic diversity/variation within populations sufficient for long-term population viability.
3. Preserve or maintain, and manage for, diversity in population identity or distinctness in the context of evolved and evolving population differentiation in the species.
4. Avoid "domestication selection" or other unintended selective effects of species or habitat management alternatives that may diminish long-term viability of evolutionarily significant units.

The population genetics component of this study will address objectives 2 and 3 most directly and contribute to development of an overlay on the HSI model to incorporate genetic diversity considerations into the framing of recommendations and priorities for future restoration activities.

3. Approach

Study Design. We will attain the goals and objectives of this project by answering a series of key questions (Table 1). These questions will be answered through field research and computer-assisted modeling as a series of specific tasks. At the end of each task, we will evaluate our data and products in a progress report that analyzes what problems were encountered, how they were addressed, and what modifications were made to the study design to better achieve the goals and objectives.

Task 1.0 Develop Habitat Suitability Model

As discussed in Section 1, several habitat relationships need further research to gain a better understanding of how the distribution of salt marsh habitat and upland areas influence the distribution of SMHM. Under this task, we will develop an HSI model that considers these relationships and determines what significant variables have the greatest influence on SMHM occurrence. The model will be constructed using data collected from Petaluma Marsh, a large salt marsh that contains a known population of SMHM. Petaluma Marsh was selected for this effort because it represents what is believed to be relatively undisturbed salt marsh habitat that still receives tidal influence. We also selected this study area to avoid potential confusion in species identification, as has been the case in Suisun Marsh (Villablanca pers. com.).

Task 1.1 Collect Baseline Data

We will collect data from both existing sources (literature, databases, and field notes) and field surveys.

A. Acquire and Review Existing Databases and Species Literature. We will query relevant databases to obtain occurrence data for SMHM in the North Bay region. As shown in Figure 3, we have derived occurrence data for SMHM from the California Natural Diversity Database (CNDDB). We will supplement this data by obtaining data from public agencies, independent consultants, universities, and other sources. Public agencies that will be contacted include the Department of Water Resources (DWR), DFG, and USFWS. This data will then be entered into the databases created in Subtask B (below).

B. Construct Project Databases. We will create three separate databases for model development. The first will track records for the region and relevant species literature. The second will be used to track field data collected under Subtask C. Lastly, a GIS database will be created for spatial information to display data and overlay with other GIS databases, such as the CNDDB, DWR data, and Environmental Science Associate's (ESA) internal GIS data sets. All databases will be updated regularly throughout the project as new information is collected.

High-resolution aerial photography for the study area will be obtained and reviewed to classify vegetation and habitats using Wildlife Habitat Relationships (WHR) and California Native Plant Society (CNPS) classification systems (other classification systems may be used where appropriate, such as the USFWS wetland classification system). Habitat polygons will be incorporated into the GIS database, which will include other data layers, such as roads, topography, land ownership, hydrology, and soils.

C. Conduct Field Surveys

Species Sampling. SMHM populations will be sampled by live trapping at 16 locations in and adjacent to Petaluma Marsh. These sampling sites will be chosen to represent four major habitat units: tidal pickleweed marsh, diked pickleweed marsh, transition to disturbed upland, and transition to upland with native vegetation. Four replicate trapping grids will be established in each of these habitat units. Grids will have an area of 1 hectare and will include 121 trap stations at 10-meter spacing. Trapping will be conducted for five consecutive nights, with one Sherman live trap at each trap station. All captured SMHM will be checked for sex, reproductive condition, and age class. They will be marked with numbered ear tags for individual identification and released at the point of capture. All 16 grids will be sampled during the summer to determine the distribution and abundance of SMHM with respect to habitat variables. Four of the grids in marsh/upland transition areas also will be sampled in winter to evaluate the importance of this habitat as escape cover during winter flooding and high tides. All data will be entered into an electronic database to be used in model construction (see Task 1.2). Field surveys will be conducted by Dr. Philip Leitner, who holds a current USFWS permit for studies of the salt marsh harvest mouse as well as a Memorandum of Understanding (MOU) with DFG for this species.

Vegetation Sampling and Other Variables. We will sample vegetation in Petaluma Marsh using a grid that overlays the grids used in SMHM sampling. We will measure and classify vegetation according to species, percent cover, and strata. Other physical

variables, such as distance to open water, salinity, soil type, and slope also will be measured. All data will be collected on standardized field forms or data recorders, to be later transferred to the HSI database.

Task 1.2 Identify Significant Variables

Once databases have been created, we will analyze the data using several statistical techniques. Although the exact methods for statistical analysis may change as a result of how data is collected and stratified, we anticipate conducting a canonical correlation analysis (COR) to determine species-environment correlation for several variables. We will use CANOCO for our analysis. Once completed, further multiple regression analysis may be warranted to define interrelationships. We also may conduct a multiple regression analysis in ArcGrid, if the data set is compatible. Significant variables identified under this step will then be used to construct the HSI model (see below). Based on past research (Shellhammer et al. 1982, Fisler 1965), significant environmental variables may include:

- Percent cover and relative height of *Salicornia*
- Distance to uplands and open water
- Polygon size of salt marsh and upland habitats
- Habitat composition of uplands
- Salinity
- Seasonal differences
- Interspecific competition

Task 1.3 Develop HSI Model

We will develop a habitat suitability index model based on the statistical analysis conducted in Task 1.2. The model will emphasize quantitative relationships between key environmental variables and species occurrence. We anticipate that the HSI model will separate habitat into four categories: high capability, medium capability, low capability, and unsuitable habitat. These categories correspond to probabilities of species occurrence; once defined they may then be used to identify potentially significant areas of SMHM activity and barriers to dispersal. The habitat relationships developed in the model will be used in conjunction with the data generated in Task 2 to develop a GIS model. The GIS model will have the ability to display this mathematically-based model graphically, thereby clearly displaying spatial relationships between habitat polygons.

Task 2.0 Population Genetics

In seeking to define the proper correspondence between ecologically suitable habitat for a species and microevolutionarily significant features related to that species' life history (e.g., mating structure, dispersal and gene flow, metapopulation structure), three lines of inquiry will be addressed in this study:

A. Have there been changes in the level and distribution of genetic variation in the Petaluma Marsh population of SMHM since the first museum collections were obtained from Sonoma County in 1908 and since more intensive studies were conducted by George Fisler in 1959.

What is the nature of those changes and how should they influence choice of restoration measures?

B. What is the degree of differentiation between the northern and southern subspecies of the SMHM and across populations of each as represented in museum specimens obtained over the past century? How should patterns of population differentiation influence restoration priorities relative to particular sites and the choice of measures to implement the objectives (1-4) listed above?

C. What is the structure of the contemporary population of SMHM in Petaluma Marsh? How does variation relate to habitat, distance between subpopulations, dispersal and gene flow, and family structure?

Question A will be addressed using mtDNA and nuclear intron sequence variation analyzed in samples taken from historical (museum specimens) and contemporary population samples collected in Petaluma Marsh and adjacent areas. Question B will be addressed using mtDNA and nuclear intron sequence data generated from museum specimens representing an array of locations surrounding San Francisco Bay. Question C will be addressed using microsatellite markers developed specifically for the SMHM. Specific methods are summarized below and detailed in Attachment A.

Task 2.1 Accession and Handling of Field Samples

Samples for DNA analysis will be collected from animals live-trapped in the field following procedures described in Attachment A. Either rump hairs pulled gently from the skin or tiny (1 mm diameter) ear punches will be taken from each animal). The use of very small tissue samples and plucked hair (von Beroldingen et al. 1987, Garza and Woodruff 1992) as sources of DNA for PCR-based studies has been well demonstrated and is standard practice in the Biosphere Genetics, Inc. (BGI) laboratory. These samples will be taken in the course of live-trapping surveys at 16 grid locations, as described in Subtask 1.1.C. In this way, it should be possible to reach a sample size of 10-20 individuals from a number of sites within Petaluma Marsh (possibly resulting in capturing over 100 individuals). Since captured mice will be marked with numbered ear tags, it will be possible to attribute each DNA sample to a unique individual. Samples will be placed in labeled vials containing 95% ethanol and will then be transported and stored at ambient temperature until receipt at the laboratory. Once in the laboratory, samples are stored at 4°C. Accessioning of samples involves inventory and recording of samples, referencing to field and other data, and creation of a database record that will be used to track the sample through the various stages of laboratory and data analysis.

It has been technically feasible for some time now to identify animals to species (Hoss et al 1992), and to assign sex and individual identity (Reed et al. 1997) using DNA isolated from fecal material. In order to conduct a preliminary trial of methods required to demonstrate and apply this approach, we will have field workers collect any fecal pellets that may be expressed by live-trapped animals so that results obtained from tissue-derived and fecal pellet-derived DNA can be compared directly for known individuals.

Task 2.2 Voucher Specimen Tissue Sample Arrangements

The primary repository of SMHM voucher specimens is the Museum of Vertebrate Zoology (MVZ) at the University of California, Berkeley. A recent query of collection records found 328 specimens of *R. r. halicoetes* and 245 specimens of *R. r. reviventris*, collected between 1908 and about 1989. The great majority of these include study skins from which tiny pieces of dried tissue can be used to obtain DNA for analysis (Thomas et al. 1990). Series from specific locations range in number from 4-10 up to nearly 100. One of the largest sets of series provided by a single collector was installed in 1959 by George Fisler and includes more than adequate numbers of specimens from the Petaluma Marsh area. Smaller series from that area were collected by MVZ founder Annie M. Alexander in 1908-1911. In combination with the samples to be collected during this study, these materials make possible the comparison of genetic diversity for Petaluma Marsh and adjacent populations of SMHM at roughly 40-year intervals since the species was first collected. In addition, museum specimens from throughout the species' range enable the overview of phylogeographic variation called for by Question B, above.

We will request tissue samples for use in this study from the Curator of Mammals, using procedures established by the MVZ. With the expectation that we will be asked to demonstrate the effectiveness of the methods to be applied to these materials in advance of approval of our request, we intend to perform initial work on samples obtained from the field at the onset of the study. In addition, we have prior work on mtDNA of other zapodid and sciurid rodents and on the Actin gene of *R. r. halicoetes* that is demonstrative.

Task 2.3 Laboratory Analyses

Laboratory work will entail: 1) extraction and purification of DNA from tissue samples, 2) screening of alternative primer and optimization of PCR and other methods involved, 3) automated sequencing of PCR products, 4) surveys of population samples by PCR and either sequencing (mtDNA and Actin sequences) or fractionation of length-varying products (microsatellite loci), 5) data recording, and 6) sequence and marker data analysis. All methods that will be employed have histories of prior use in the literature (see Attachment A) and in the experience of those participating in this proposal (Riggs et al. 1997, Riggs 1998, Villablanca 1993). Further detail on methods that will be employed in each of these steps is provided in Attachment A.

Task 2.4 Comparative Data Analysis

Phylogeographic and population genetic analytic approaches will be used to compare data sets obtained from museum specimens and from field sampling to address the key questions posed in this study. Contemporary samples from Petaluma Marsh will be compared with historical (museum voucher) samples using mtDNA, nuclear intron, and microsatellite data to determine how genetic diversity may have changed over the past 90 years. Historical samples from throughout the range of the species will be compared to delineate the extent of genetic variation in *R. raviventris* in space and time. We anticipate that the collaboration with Dr. Francis

Villablanca and exchange of primers and methods also may make possible a comparison of the population in Petaluma Marsh with the one in Suisun Marsh being studied by Dr. Villablanca.

Task 2.5 Determination of Restoration Model Parameters

With production of the genetic data described above, two kinds of parameters useful to development of a restoration model can be derived. Effective population sizes (N_e), corresponding to population number and deviations from Hardy-Weinberg assumptions (due to age and family structure and population subdivision) will be determined. In addition, genetic information necessary to incorporation of metapopulation-maintenance and restoration-source recommendations will be available. Both information and approaches from the genetics effort will feed into the model development effort at intervals throughout the study.

Task 3.0 Determine Spatial Requirements

Task 3.1 Develop GIS Model

Using the HSI model created under Task 1.0 and the results of Task 2.5, we will develop a GIS model in ArcInfo (and the associated extension ArcGrid) to map suitable habitat in Petaluma Marsh. While the type of data collected will ultimately affect specific model design and methods, we anticipate constructing a script in Arc Macro Language (AML) to derive new data set from the GIS data library constructed in Task 1.1. The script will be constructed as a series of reselects, joins, and dissolves that are based on the variables identified in the HSI model. For example, we may reselect from the vegetation layer all *Salicornia* polygons with greater than 50% cover and join those polygons with a layer of upland habitats that are buffered at regular distances. The polygons will then be dissolved to form a new data set (i.e., polygons with high pickleweed cover *and* within **x** distance of uplands). This process will be repeated for all variables included in the HSI model and for each habitat capability category (except unsuitable, which will be considered the null or universal polygon). Figure 4 gives a graphical representation of this process.

Task 3.2 Evaluate Model

Once the GIS model has been developed, we will evaluate the model under two environments. Our first statistical test will be to overlay the habitat capability layers constructed for Petaluma Marsh over the occurrence data collected by Dr. Leitner. Should the model show a poor degree of correspondence with this data, we will reevaluate the parameters used in the model to determine if variables should have been weighted differently or if interrelationships were misinterpreted. Once the model is shown to be statistically accurate, we will conduct targeted surveys in the marsh to further validate the model. These surveys will target each of the habitat categories (high, medium, low, and non-) to determine their level of accuracy. This also may provide information on what may be expected in terms of population density within each of the habitat categories.

Once the above work within Petaluma Marsh is complete, we will recompile the GIS script to apply it to a different region. Although selection of a specific test region in the North Bay will be dependent on a number of factors (primarily the availability of suitable GIS data), we

anticipate that Suisun Marsh may prove to be an excellent candidate for testing. Suisun Marsh has a complete GIS data set associated with it, and occurrence data is easily accessible. Similar to the exercise performed for Petaluma Marsh, we will overlay the derived habitat capability layers over occurrence data to determine the degree of overlap, thereby testing the model in a different geographical region.

Task 3.3 Identify Gaps in Habitat Distribution

As a final task to determine spatial distribution interrelationships, we will identify potential gaps in habitat distribution within the study area and selected test area. Polygon distribution data will be compared with the genetic analysis conducted under Task 2.0 and the documented home range for SMHM to determine if there is a relationship between genetic variation in the metapopulation and the spatial distribution of habitat. This information will be used to conduct Task 4.0.

Task 4.0 Evaluate Restoration Priorities

Task 4.1 Correlate Model to Habitat and Population Distribution

We hypothesize that genetic variation may be significantly related to both habitat quality and distribution. While the modeling and genetic analysis may not ultimately provide a clear answer to this question, we do anticipate that one of two scenarios may develop:

1. (A) In areas of medium capability habitat within a matrix of medium or low capability habitat, genetic variation is high, and (B) in areas of high or medium capability habitat within a matrix of low to unsuitable habitat, genetic variation is low. These relationships may indicate that the spatial distribution of habitat has a direct correspondence to genetic diversity at the population level. In other words, the matrix should be emphasized in species management.

2. (A) In areas of high capability habitat within a matrix of low capability or unsuitable habitat genetic diversity is high, and (B) in areas absent of high capability habitat within a matrix of medium or low capability habitat, genetic diversity is low. This may indicate that habitat quality within occupied polygons has the greatest influence on genetic diversity.

Each of the above scenarios has different implications for conservation and restoration. Under Scenario 1, linkages to other habitat polygons (the matrix between conservation areas) will be emphasized. Under Scenario 2, maintaining large polygons of high capability habitat around existing and potential populations will be emphasized. Should a definitive relationship be identified, we will use the results of this analysis in formulating recommendation for Task 4.2.

Task 4.2 Prioritize Habitat Polygons for Restoration

Once we have determined the relationship between population genetics and habitat distribution (if any), we will identify specific polygons within Petaluma Marsh and the test area that should be targeted for restoration. Depending on the results obtained under Task 4.1, this task may

target the matrix (i.e., dispersal habitat) for restoration, or it may target high capability habitat and associated upland refugia, or a combination of both to varying levels. The end goal of this project is to provide a model that may be applied to multiple North Bay regions and will yield consistent results in terms of identifying restoration priorities. Once specific polygons have been identified, we will make management recommendations for SMHM in a final technical report. The report will emphasize the metapopulation dynamics of SMHM, how habitat should be managed spatially at the landscape level, and identify in what aspects SMHM may be most vulnerable to a reduction in genetic variation.

4. Feasibility

Approach. Our approach considers two factors important to SMHM conservation and recovery in a cost-effective, timely manner: 1) determining the fine structure of SMHM population genetics, and 2) identifying spatially priorities for restoration projects that are most likely to benefit the species. Our three-year study period and budget is projected to sufficiently allow for delays due to weather conditions or the need to acquire additional data. Our sampling schedule allows adequate time for such delays or acquisition needs.

Some tasks (particularly Task 4.0) will be dependent on the success of both the model and the genetic analysis. Therefore, the nature of this task may change significantly to reflect the results obtained under Tasks 2.0 and 3.0. For example, some environmental variables not previously considered significant may prove to be important, thereby driving the model in unexpected directions. To account for this, we will closely monitor our progress as tasks are completed, and produce progress reports to determine if overall goals or objectives are in need of revision.

All of the sample handling, DNA extraction and purification, DNA amplification, and DNA fractionation techniques proposed for use in this study have been used extensively in published and unpublished work by many laboratories. Scientists associated with the laboratory managed by Dr. Riggs have direct experience with all of these techniques. We will have access to special expertise required to address any problems that may arise through a permanent panel of scientific and technical advisors available to Dr. Riggs, as well as through several of the scientific reviewers named for this study.

Required Permits and Agreements. Dr. Leitner, who holds a current USFWS permit for studies of SMHM as well as an MOU with DFG, will conduct all field surveys for this species. Dr. Niall McCarten will conduct all vegetative sampling, while Dr. Riggs will be responsible for all DNA laboratory analysis.

Most sampling will be conducted within the Petaluma Marsh Wildlife Area, which is managed by the DFG. We have had initial discussions with DFG on this project, and will work closely with DFG staff in the sampling effort. Areas outside of the Petaluma Marsh Wildlife Area will be sampled on a case-by-case basis, contingent on acquiring permission from individual landowners. Sampling outside of the marsh is not considered critical to the success of this project, as the marsh provides sufficient area and habitat types for sampling.

5. Performance Measures

We will monitor performance throughout the projects by monitoring the achievement at specific milestones. For each of the tasks described in Section 3.0, we will have milestones that must be completed prior to initiating subsequent tasks. At each milestone, we will evaluate the products and data acquired in a report to determine and document how much progress towards the goals and objectives is being made. Throughout the process, we will consult with the USFWS, DFG, and associate researchers to ensure that data and methodologies are kept current.

Progress on the genetics component of this study is easily quantifiable in terms of numbers of samples processed through each stage of processing: sample collection, accession, and DNA extraction and purification. For analytic activities, a useful progress parameter is the number of markers resolved via PCR, fractionation, data recording, and analysis. Targets will be established relative to the work plan at the beginning of the study, and progress toward those targets will be identified in each quarterly report.

6. Data Handling and Storage

A complete set of originals and copies of all data collected will be maintained by ESA. Data will be stored in Microsoft Access and ArcInfo format. Because of the sensitive nature of this data, it will only be available through contacting the appropriate resource agency (USFWS or DFG). Field survey data on federal and state-listed and other special-status plants and wildlife collected under authorized permits will be entered into the required federal forms for the USFWS and Field Survey Forms for the CNDDB. Copies of all data collected will be included in biological baseline studies and monitoring reports. This data will be closely shared with the DFG for the use at the Petaluma Marsh Wildlife Area.

Data acquired from laboratory analyses will be maintained in databases associated with the program Phoretix 1D (Phoretix International Ltd., Birmingham, UK). Data is exportable via Excel files to database systems maintained by ESA and DFG. Reports will be provided in one or more of the following forms as preferred: hard copy; Microsoft Word, PDF, and/or HTML files on CD-ROM; e-mail attachment or web accessible files.

7. Expected Products and Outcomes

We anticipate the following reports and products:

- Habitat Model Technical Report (updated after each task)
- Population Genetics Technical Report (updated after each task)
- GIS Script in AML (or similar scripting language)
- Occurrence Data/Maps for Study Area
- Habitat Capability GIS Data Sets for Petaluma Marsh and other test area
- Restoration Priorities Maps and Management Recommendations Report

Most products will be available in both hard copy and electronic (PDF) formats.

8. Work Schedule

The proposed project will be completed in three years. The proposed schedule takes into consideration potential problems with data collection techniques; participation by various local, state, and federal agencies; and minor changes in climate. The proposed activities are all designed to include activities that can occur during different seasons. The most important seasonal timing is the sequence of up to three late fall-winter surveys. Three seasons of survey during the flood season will allow for a complete analysis of the importance of uplands to the species. If one or more years are drier than normal, we will adjust the study to measure the biological and habitat variables that can be measured under drought conditions. The model and associated restoration recommendations will be written to address the conditions under which the documents were developed.

Our study is modular in design to easily separate some tasks. For example, Task 2 may be considered a complete study in itself, as may Tasks 1 and 3. Task 4 is the only task that relies on the completion of all tasks prior to it. Each task may be funded separately or even by subtask.

B. Applicability to CALFED ERP and Science Program Goals and Implementation Plan and CVPIA Priorities

1. ERP, Science Program and CVPIA Priorities

CALFED MSCS Milestones

Suisun Marsh and North San Francisco Bay Habitat Milestones. Acquire land needed for tidal restoration and complete the steps to restore wetlands to tidal action. This project will assist in focusing the limited resources available for saline emergent wetland restoration towards the habitat with the greatest potential to be effectively restored. This project will also identify the most important components missing for land acquired for restoration.

CALFED MSCS

Species Goal for Salt Marsh Harvest Mouse. Contribute to the recovery of SMHM by implementing some of the actions deemed necessary to recover the species populations within the MSCS focus area. The USFWS joint Recovery Plan for California clapper rail and SMHM states that "(p)rotecting these species will require the protection and enhancement of existing marshes, the restoration of former habitat, and additional research on their habitat requirements and population trends, especially in San Pablo Bay and Suisun Marsh" (USFWS 1984). One of the specific conservation needs is the development of management plans that include specific management guidance. A key component to developing management guidance is to determine the habitat requirements of salt marsh harvest mouse in tidal and brackish marshes.

ERP Strategic Goals and Objectives

Goal 1: Achieve recovery of at-risk native species dependent on the Delta and Suisun Bay as the first step toward establishing large, self-sustaining populations of these species. There is

considerable uncertainty about how best to facilitate the recovery of these species. ERP actions must address the immediate needs of at-risk species as well as gain additional information about how they respond to modifications to ecosystem functions and processes. This study will improve our understanding of the best methods for restoring SMHM and their habitat.

Goal 4: Protect and/or restore functional habitat types in the Bay-Delta estuary and its watershed for ecological and public values such as supporting species and biotic communities, ecological processes, recreation, scientific research and aesthetics. Though the importance of restoring additional habitats is not debated. This study will help prioritize the difficult choices ahead regarding the relative importance of restoring different habitat types on regional and local scales. This study will also meet a pressing need to develop better tools to make decisions on how to manage and restore SMHM habitat.

Regional Goals and Objectives

BR-1: Restore Wetlands in critical areas throughout the Bay, either via new projects or improvements that add or help sustain existing projects. The proposed project would facilitate restoration of several wetland types emphasized by this goal, including tidal marsh and tidally muted marsh.

BR-2: Restore uplands in key areas of Suisun Marsh and San Pablo Bay. This project may identify the need to protect and restore upland habitat for SMHM escape cover, thereby prioritizing restoration in these locations.

CALFED Science Program Goals

Restoration is a new science and uncertainty exists about how to most effectively restore communities and ecological function, what communities might result from restoration efforts, and how to sustain restoration. The long-term goal of the CALFED Science Program is to progressively build a body of knowledge that will continually improve the effectiveness of restoration actions and that will allow the CALFED Program to track restoration progress. The priorities of the Science Program include:

- *Develop performance measures.* Scientific studies are needed to demonstrate and establish performance measure monitoring.

- *Build population models for at-risk species.* This requires knowledge of life history, environmental requirements and biology of at-risk species, and ultimately developing reliable models of population processes.

- *Establish integrated science programs in complicated field settings*. It is the goal of the Science Program to establish intensive site-, multi-site-, or watershed-specific interdisciplinary programs in every region.

- *Advance the scientific basis of regulatory activities*. The present state of knowledge is imperfect and uncertainties exist in the science that is applied. It is critical to continually address, explain, and advance the knowledge that can be applied to management, with the

goal of adapting regulatory activities as the knowledge changes. Addressing the uncertainties in the science used for management is an important goal of the CALFED Science Program.

- *Take advantage of existing data*. Projects are encouraged that develop questions that can be addressed by interpreting existing data and that can build from that data to develop indicators and better understanding of processes, species, and communities.

CVPIA Goals

Contribute to the State of California's interim and long-term efforts to protect the San Francisco Bay and Sacramento-San Joaquin Delta Estuary. The Central Valley Project Conservation Program implements projects to protect, restore, and enhance federal threatened or endangered species, other special-status species, and their habitat in areas directly or indirectly affected by the CVP.

2. Relationship to Other Ecosystem Restoration Projects

The proposed project will provide a foundation for restoration projects in the North Bay that wish to consider the specific habitat requirements of SMHM. Several large restoration projects (some that are required for mitigation under NEPA/CEQA) are planned for the region, and this project may be used in those planning efforts to ensure that this species specific habitat needs are taken into account.

3. Requests for Next-Phase Finding

Not Applicable.

4. Previous Recipeints of CALFED Program CVPIA Finding

Not Applicable.

5. System-Wide Ecosystem Benefits

The proposed model and genetic information may be used throughout the North Bay Region. The model also may help address current management issues that surround this species, such as differentiating from closely associated species (*R. megalotis*) or identifying the importance of uplands spatially. The model may ultimately be used to identify potential recovery options at the landscape level through modeling habitat throughout the North Bay Region.

6. Additional Information for Proposals Containing Land Acquisitions

Not Applicable.

C. Qualifications

Erich Fischer is a senior wildlife biologist with ESA who serves as a Project Manager and Technical Analyst for a variety of projects. He received his B.A. in Biological Sciences (conservation biology) from California State University, Sacramento. Mr. Fischer has over 11 years of experience in conducting field studies, modeling on GIS systems, and preparing technical and regulatory reports. He is certified in habitat delineation techniques, habitat evaluation procedures, and several remote-sensing techniques. He has successfully developed habitat suitability index models and associated GIS models for several special-status species in California, including mesocarnivores, raptors, reptiles, and amphibians. Many of the models were subsequently used by land management agencies (such as the U.S. Forest Service) to assist in species management and in conducting impact analyses. Mr. Fischer will act as the Project Manager for the HSI and GIS models, ensure coordination with federal and state agencies, and maintain communication with DFG staff. He will ensure that all goals and objectives are met.

Philip Leitner is a wildlife biologist with 27 years of experience as an independent consultant in biological resource conservation. Dr. Leitner is a sole proprietor whose clients have included a diverse range of federal, state, and local agencies as well as research institutes, corporations, and non-profit organizations. He has extensive experience with analysis of biological resource issues through the CEQA and NEPA processes and has prepared the biology sections of over 50 environmental impact documents. Dr. Leitner has expertise in wildlife field surveys and inventories, habitat evaluation, impact assessment, mitigation planning, compliance monitoring, resource management planning, and expert testimony. He has developed excellent working relationships with staff of important regulatory and resource management agencies, including California Department of Fish and Game, California Department of Water Resources, California Energy Commission, U.S. Fish and Wildlife Service, U.S. Bureau of Land Management, and U.S. Forest Service. He has special interest in threatened and endangered wildlife species of California and has conducted monitoring and research studies of a number of sensitive species. He currently holds state and federal permits for field investigations of several listed species, including the Mohave ground squirrel (*Spermophilus mohavensis*) and salt marsh harvest mouse (*Reithrodontomys raviventris*). Dr. Leitner will act as the project manager for sampling salt marsh harvest mouse.

Niall McCarten is senior biologist with ESA and Research Associate with the Section of Plant Biology at UC Davis, and the UC Jepson Herbarium at UC Berkeley. He received his B.A. in botany at UC Santa Barbara, M.A. in Ecology and Systematics at San Francisco State University, and Ph.D. in botany at UC Berkeley. He is a nationally recognized botanist and plant ecologist with peer reviewed papers and conference presentations on rare and endangered plants, wetlands ecology and monitoring. He has served as the project manager on many large projects involving teams of scientists, resource agency staff, and consultants. He was one of the few non-public agency scientists asked to participate in the development of the original CALFED ERP plan, and to participate in the development of the CALFED MSCS. Dr. McCarten will be act as the project manager for collecting all vegetation data for the project and oversee statistical methods used in the HSI model.

Lawrence Riggs is a population geneticist and evolutionary biologist who has been working at the interface between research and application for the past 20 years. He received his A.B. from Dartmouth College. After beginning graduate work at the University of Colorado, Boulder, he moved to the University of California at Berkeley, where he trained with then Curator of Mammals, Dr. William Z. Lidicker, and conducted dissertation research using allozymes to examine the microevolutionary changes occuring in conjuction with dispersal and and other demograhic events in experimental populations of *Microtus californicus*. He received his Ph.D. in Zoology in 1979. He taught at the University of California, Santa Barbara, and worked with the U.S. Fish and Wildlife Service and the National Council on Gene Resources' California Gene Resources Program before consolidating his independent consulting activities under the name of Genetic Resource Consultants. He was a co-founder of Biosphere Genetics, Inc. (BGI) in 1991 and has been a Principle Investigator on projects applying genetic information and a variety of molecular marker techniques to conservation, restoration, and resource management for the past 10 years. He currently serves as the company's president and CEO. Dr. Riggs will act as project manager for analyzing the genetic variation of salt marsh harvest mouse.

Francis Villablanca is a molecular ecologist and biosystematist with research interests in molecuclar and organismal evolution, phylogeography, and conservation genetics. He received his B.S. from California Polytechnic State University and his Ph.D. at the University of California, Berkeley. At Berkeley he was a student in the Museum of Vertebrate Zoology and was associated with the laboratory of the late Dr. Alan Wilson. He participated in pioneering work using the polymerase chain reaction to advance molecular methods for applications in systematics, population genetics, and evolutionary studies and co-authored several landmark papers demonstrating these advances. Dr. Villablanca is currently Assistant Professor in the Biological Sciences Department at California Polytechnic State University where he teaches and advises students in areas related to his research interests. His research program currently includes an effort to distinguish *Reithrodontomys raviventris* from its more common relative *R. megalotis* and hybridization and phylogenetic issues using several complementary molecular methods. Dr. Villablanca will collaborate with BGI staff in the population genetics analysis.

Chris Rogers is a wetlands and plant ecologist with ESA. He has over 12 years experience conducting habitat assessments, endangered species evaluations, preparation of environmental documentation and permitting applications, restoration and mitigation planning, and construction monitoring. He received his B.A. in Biology (emphasis plant ecology) at San Francisco State University. Mr. Rogers has applied his specific experience to numerous projects across the State of California. His restoration experience includes preparing restoration and revegetation plans for Alhambra Creek in Martinez involving extensive planting of a native cordgrass marsh, developing long-term marsh and riparian habitat restoration. In addition, Mr. Rogers has conducted numerous site assessments of wetlands and streams and feasibility studies for restoration, enhancement and water treatment applications. Mr. Rogers will be an integral contributor to the vegetation sampling team and assist in designing restoration priorities.

Thomas Leeman is a wildlife biologist and ornithologist with ESA in Sacramento, California. He received his B.S. in Biology from the University of California at Davis and his M.S. in Natural Resources with a Wildlife emphasis from Humboldt State University. He has ten years experience coordinating and conducting field studies in wetland, upland and riverine habitats.

Mr. Leeman will be an integral contributor to the vegetation sampling and SMHM sampling team and assist in designing restoration priorities.

D. Cost

1. Budget
The total estimate cost for the three-year project will be $820,726.00.

2. Cost Sharing
Not applicapble.

E. Local Involvement

This project has support from the CDFG at multiple staff levels in the North Bay Region. As stated previously, we will work closely with CDFG staff in the Petaluma Marsh Wildlife Area to ensure project activities do not conflict with ongoing activities.

F. Compliance with Standard Terms and Conditions

We will comply with the standard State and Federal contract terms as described in the 2002 ERP Proposal Solicitation Package.

G. Literature Cited

Botti, F., D. Warenycia, D. Becker. 1986. Utilization by salt marsh harvest mice *Reithrodontomys raviventris halicoetes* of a non-pickleweed marsh. Calif. Fish and Game 72:62-64.

Burkey, T.V. 1989. Extinction in nature reserves: The effect of fragmentation and the importance of migration between reserve fragments. Oikos 55:75-81.

Fahrig, L., and G. Merriam. 1985. Habitat patch connectivity and population survival. Ecology 66:1762-1768.

Fisler, G. F. 1965. Adaptations and speciation in harvest mice of the marshes of San Francisco Bay. University of California Publications in Zoology 77:1-108.

Garza, J. C. and D. S. Woodruff. 1992. A phylogenetic study of the gibbons (*Hylobates*) using DNA obtained noninvasively from hair. Molecular Phylogenetics and Evolution 1(3):202-210.

Geissel, W., H. Shellhammer, and H. T. Harvey. The ecology of the salt-marsh harvest mouse (*Reithrodontomys raviventris*) in a diked salt marsh.

Hoss, M., M. Kohn, S. Paabo, F. Knauer, and W. Schroder. 1992. Excrement analysis by PCR. Nature 359:199.

Merriam, G. and A. Lanoue. 1990. Corridor use by small mammals: Field measurements for three experimental types of *Peromyscus leucopus*. Landscape Ecology 4:123-131.

Noss, R. F., and L. D. Harris. 1986. Nodes, networks, and MUMs: Preserving diversity at all scales. Environmental Management 10:299-309.

Reed, J.Z., D. J. Tollit, P.M. Thompson and W. Amos. 1997. Molecular scatology: The use of molecular genetic analysis to assign species, sex and individual identity to seal feces. Molecular Ecology 6(3):225-234.

Riggs, L.A. 1998. Evaluating genetic identity and distinctness of jumping mice (Zapus) from F.E. Warren Air Force Base using mitochondrial DNA sequence information. Completion Report, Contract No.F48608-E-041, U.S. Air Force.

Riggs, L. A., J. M. Dempcy and C. Orrego. 1997. Evaluating distinctness and evolutionary significance of Preble's meadow jumping mouse (*Zapus hudsonius preblei*): Phylogeography of mitochondrial DNA non-coding region variation. Final Report prepared for the Colorado Division of Wildlife, Denver Colorado.

Shaffer, M. L. 1990. Population viability analysis. Conservation Biology 1:39-40.

Shellhammer, H.S., R. Jackson, W. Davilla, A. M. Gilroy, H. T. Harvey, and L. Simons. 1982. Habitat preferences of salt marsh harvest mice (*Reithrodontomys raviventris*). Wasmann J. Biol. 40:102-114.

Shellhammer, H. S. 1982. Reithrodontomys raviventris. Mamm. Species. 169:1-3.

Thomas, W.K., S. Paabo, F.X. Villablanca, and A.C. Wilson. 1990. Spatial and Temporal Continuity of Kangaroo Rat Populations Shown by Sequencing Mitochondrial DNA from Museum Specimens. *Journal of Molecular Evolution*. 31:101-112

USFWS. 1984. Salt Marsh Harvest Mouse and California Clapper Rail Recover Plan. USFWS, Portland, Oregon.

Villablanca, F.X. 1993. Evolutionary Analysis: Spatial and Temporal Aspects of Populations Revealed by Mt. DNA. *In* Ancient DNA. B. Heermann and S. Hummel (Ed.). Springer-Verlag, N.Y. pp. 31-58.

von Beroldingen, C. H., R., G. Higuchi, G. F. Sensabaugh, and H. A. Erlich. 1987. Analysis of enzymatically amplified HLA-DQalpha DNA from single human hairs. Amer. J. of Human Genetics 41:725.

Wondolleck, J. T., W. Zolan, and G. L. Stevens. 1976. A population study of harvest mice in the Palo Alto Salt Marsh. Wasmann Journal of Biology 34:52-64.

TABLE 1. LIST OF TASKS, ACTIVITIES AND KEY QUESTIONS

Task/Activity	**Key Questions**
TASK 1: DEVELOP HABITAT SUITABILITY MODEL Task 1.1: Collect Baseline Data Task 1.2: Identify Significant Variables Task 1.3: Develop HSI Model	*How do habitat quality and quantity affect species distribution?*
TASK 2: POPULATION GENETICS Task 2.1: Accession and Handling of Field Samples Task 2.2: Voucher Specimen Arrangements Task 2.3: Laboratory Analysis Task 2.4: Comparative Data Analysis Task 2.5: Determine Restoration Model Parameters	*How has genetic variation changed over the past 90+ years? How should population differentiation influence restoration priorities? What is the structure of the contemporary population of SMHM in Petaluma Marsh and how does variation relate to habitat selection and distribution?*
TASK 3: DETERMINE SPATIAL REQUIREMENTS Task 3.1: Develop GIS Model Task 3.2: Evaluate Model Task 3.3: Identify Key Gaps in Habitat Distribution	*How does habitat distribution and interrelationships affect population distribution and genetic variation?*
TASK 4: EVALUATE RESTORATION PRIORITIES Task 4.1: Correlate Model to Habitat and Population Distribution Task 4.2: Identify Restoration Polygons based upon Specific Variables	*Where should land managers and resource agencies target efforts for restoration to ensure genetic diversity is conserved?*

TABLE 2. PROPOSED WORK SCHEDULE

Task/Activity	Start Date	End Date
TASK 1: DEVELOP HABITAT SUITABILITY MODEL		
Task 1.1: Collect Baseline Data	August 2002	August 2003
Task 1.2: Identify Significant Variables	August 2003	October 2003
Task 1.3: Develop HSI Model	October 2003	November 2003
TASK 2: POPULATION GENETICS		
Task 2.1: Accession and Handling of Samples from the Field	August 2002	August 2003
Task 2.2: Arrangements for Voucher Specimens	August 2002	May 2003
Task 2.3: Laboratory Analysis	November 2002	December 2004
Task 2.4: Comparative Data Analysis	December 2003	May 2005
Task 2.5: Determine Restoration Model Parameters	February 2004	May 2005
TASK 3: DETERMINE SPATIAL REQUIREMENTS		
Task 3.1: Develop GIS Model	November 2003	February 2004
Task 3.2: Evaluate Model	February 2004	February 2005
Task 3.3: Identify Potential Gaps in Habitat Distribution	February 2005	March 2005
TASK 4: EVALUATE RESTORATION PRIORITIES		
Task 4.1: Correlate Model to Habitat and Population Distribution	May 2005	June 2005
Task 4.2: Identify Restoration Polygons based upon Specific Variables	June 2005	August 2005

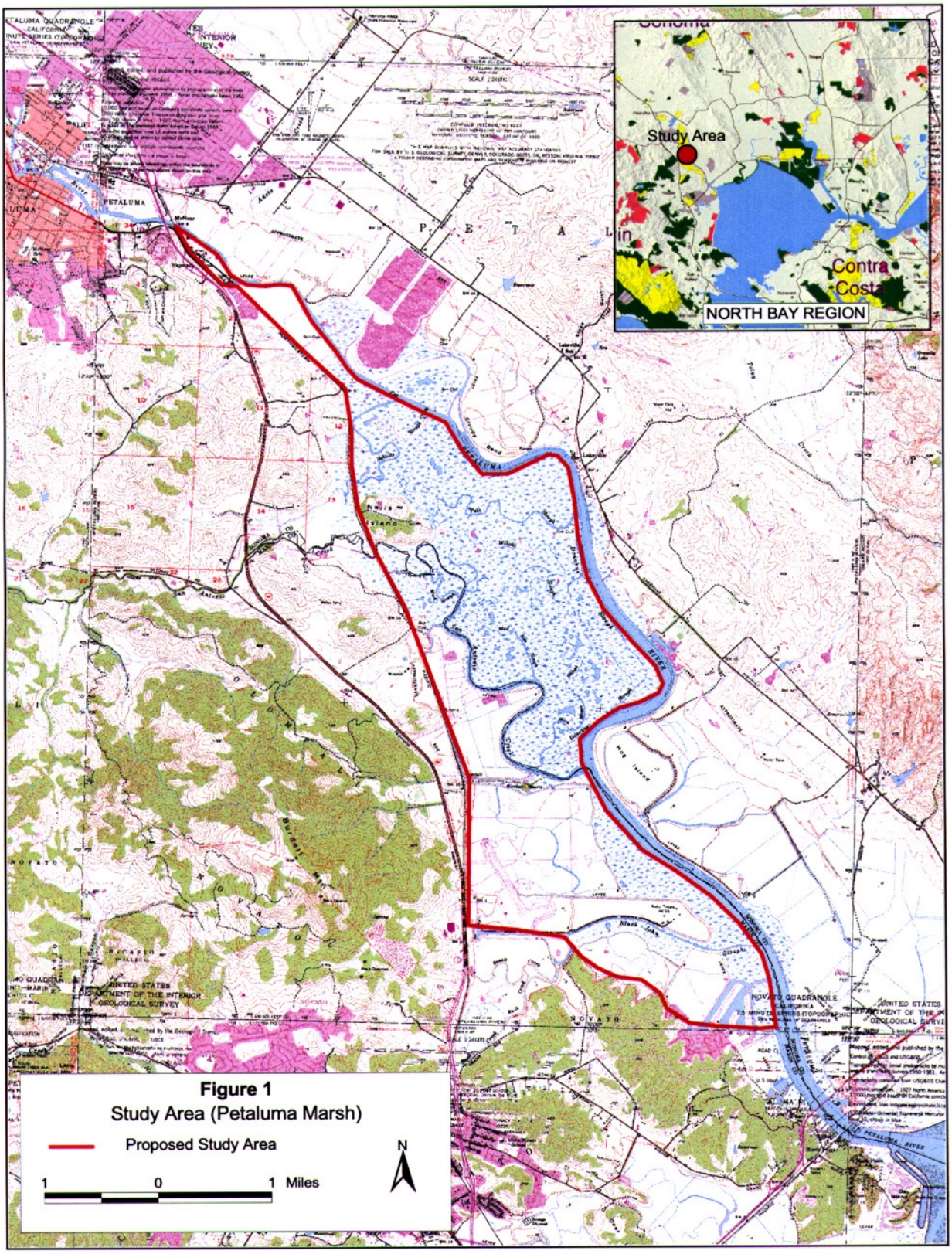
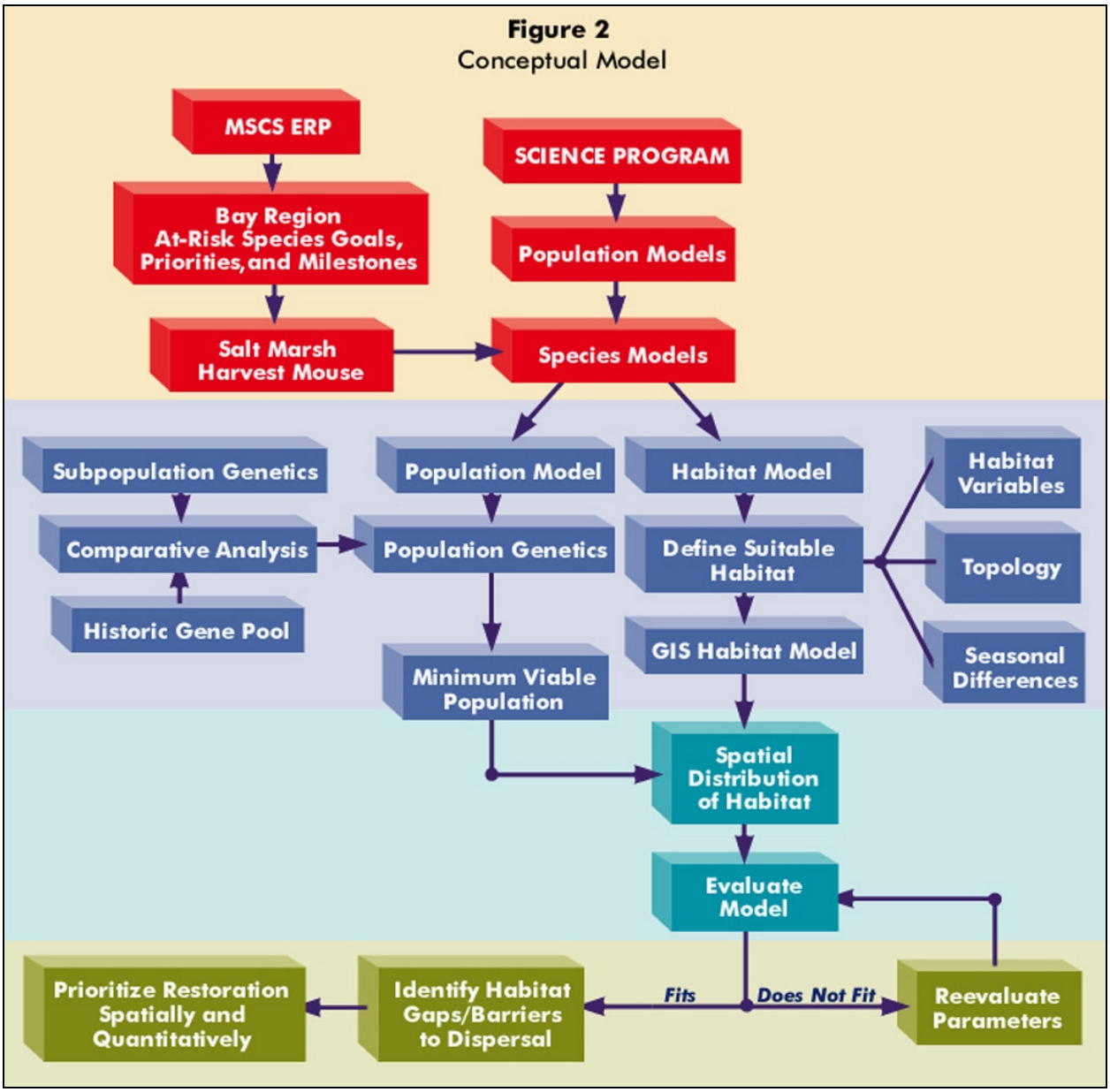


Figure 1
Study Area (Petaluma Marsh)

Figure 2
Conceptual Model



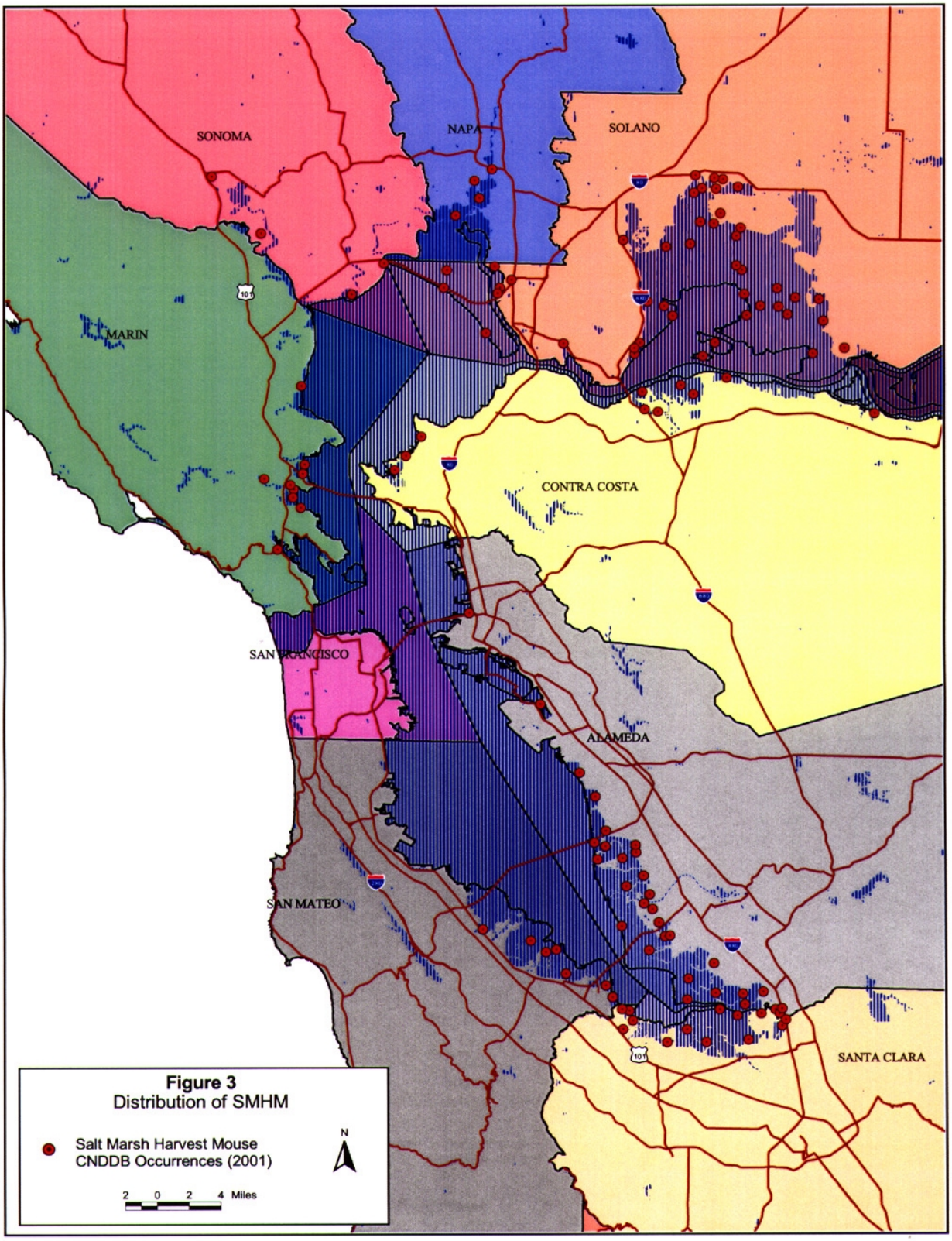
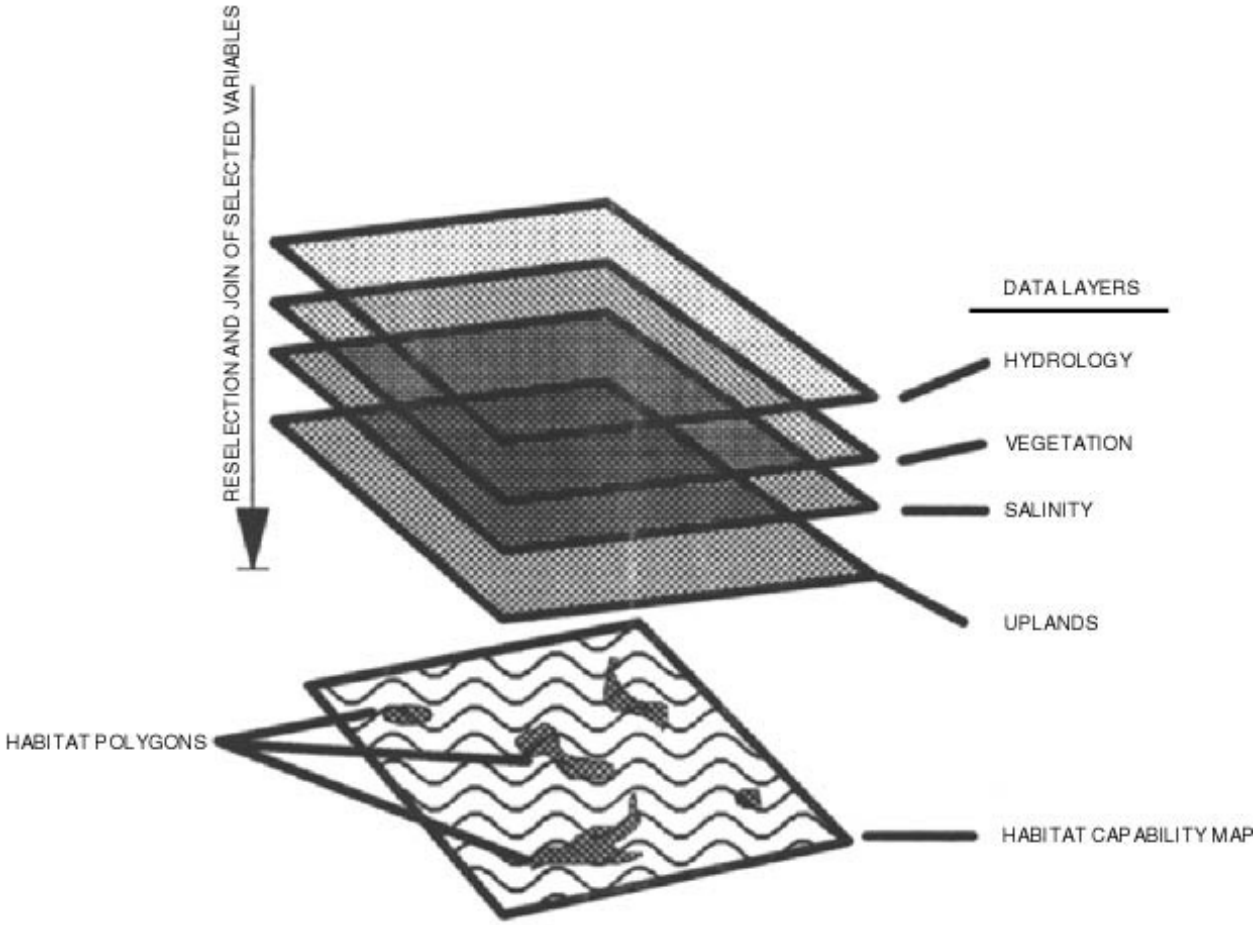

SONOMA
NAPA
SOLANO
MARIN
CONTRA COSTA
SAN FRANCISCO
ALAMEDA
SAN MATEO
SANTA CLARA
101
Figure 3
Distribution of SMHM
Salt Marsh Harvest Mouse
CNDDB Occurrences (2001)
N
2 0 2 4 Miles

Figure 4

Example of GIS Data Integration Process



Attachment A

Additional Detail on Sampling and Laboratory Methods

DNA Extraction & Purification

Prior work on mtDNA sequences conducted by BGI has used a simple extraction technique know as the hair lysis buffer (HLB) method to obtain ample quantities of DNA from museum tissue samples, ear punches, and plucked hair samples. Confirmation that amplified fragments targeted in mitochondrial DNA were not contaminated by similar sequences in nucelar DNA also found in these genomic DNA extracts was provided by registering sequence data obtained from these extracts to conserved regions known to be invariant in mtDNA genomes of widely divergent taxa. In the first stages of work to identify target sequences for *R. raviventris* it may be necessary/advisable to prepare DNA using a mitochondrial miniprep procedure. However, our experience has been that the HLB method should be effective in combination with properly optimized PCR conditions to obtain clean mtDNA-origin fragments for target sequences in population survey analyses. Genomic DNA extracts obtained by the HLB method are also appropriate for amplification of nuclear intron and microsatellite regions.

Mitochondrial DNA Analysis

Mitochondrial DNA has been used extensively in the study of animal populations, particularly since the demonstration of methods employing the polymerase chain reaction (Kocher et al. 1989). A collaborator in this propsal, Dr. Francis Villablanca, was among the first to demonstrate that PCR methods could obtain phylogenetically useful information from mtDNA preserved in the tissues of museum specimens (Thomas et al. 1990). The value of mtDNA analysis to conservation biology was recognized almost immediately (Moritz 1994) and related methods continue to be applied to a wide array of animal taxa for a variety of purposes (Edwards 1993, Petri et al. 1997, Chenoweth et al. 1998, and many others).

Our own previous works suggests several regions in mtDNA that may be of interest to this investigation. One portion of the non-coding region that may be a good candidate for assay in both museum specimen and live-trap sampled material is a sequence approximatelyy 450 bp long bracketed by primers PRPRO-H and TDKD (see Fig. 1). This sequence revealed subspecies- and population-level variation in studies of *Zapus hudsonius*, a species listed as threatened in Colorado (Riggs et al. 1997, Riggs 1997). Because work on various animal species has found different levels of variation in this and other portions of the mtDNA genome it may be advisable to use a primer pair such as ProC and HuPhe RevH29 to amplify essentially the entirety of the non-coding region in a number of representative samples and sequence the products. Alignment of the sequences and comparison to baseline non-coding region sequence for rat or mouse would help identify the most productive portions of the non-coding region to target with available or newly developed primers for assay of informative variation in population samples.

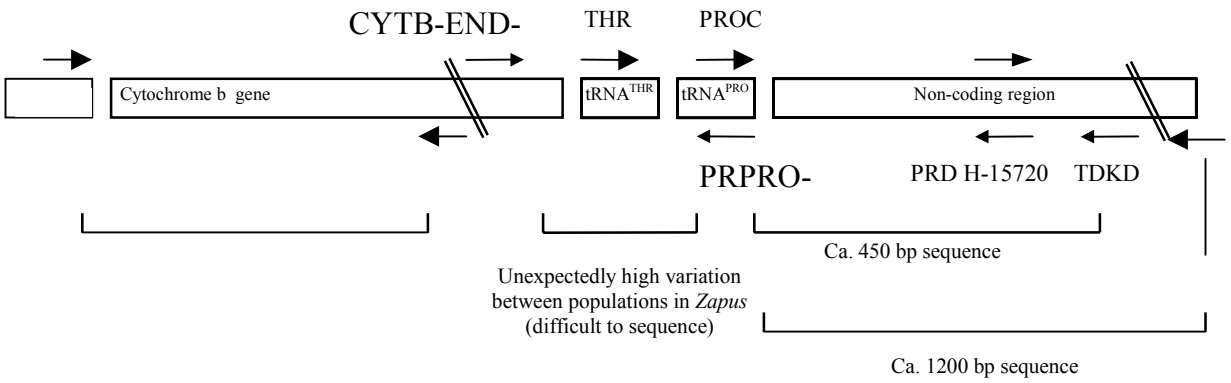


Fig. 1. Relative positions of the primers and the regions they amplify in mitochondrial DNA. The ca. 450 bp long sequence targeted by primers labeled PRPRO-H and CYTB-END-L is one candidate for analysis in this study. The primer combination PROC-HuPhe RevH29 may be used to amplify the entire non-coding region for sequencing and comparison in a subset of samples in order to identify maximally informative variation in shorter regions more easily assayed in population samples. The relative positions of coding and non-coding regions in the mitochondrial DNA are indicated by labeled areas.

Nuclear Intron Analysis

Introns are highly polymorphic regions of the nuclear genome (spacers between protein coding blocks) and have a history of use in conservation genetics (Palumbi and Baker 1996). Variation in intronic regions of nuclear DNA can be contrasted with variation in mitochondrial DNA (known to be maternally inherited) to illuminate the role of male dispersal and gene flow in populations.

Our study will generate data comparable to what is already being generated for the Actin locus of the Suisun Marsh population of *R. raviventris* (Villablanca, personal communication). PCR amplifications will target intron 1 of the nuclear Actin locus (Palumbi 1996) using universal primers already available for Actin and demonstrated to amplify a diversity of organisms from gray whales (Palumbi and Baker 1996) to black-footed ferrets and kangaroo rats as well as harvest mice (Villablanca personal communication). The amplification of nuclear DNA and cloning that is required in order to separate alleles from diploid organisms is routinely done in Dr. Villablanca's lab (Villablanca et. al. 1998), and this work will be conducted by BGI in collaboration with him and his graduate students. Dr. Villablanca has developed a simple restriction digest assay that can confirm proper identification of salt marsh harvest mice (*R. raviventris vis a vis R. magalotis*) based on his prior phylogenetic analysis of variation in a portion of the 1600 bp long Actin gene. Actin gene sequence data acquired in this study will extend testing of this assay as well as provide phylogeographic data to compare and contrast with mitochondrial DNA data.

The data analysis for nuclear fragments is substantially different from that of mitochondrial DNA since regions of nuclear DNA can recombine. Phylogeographic analysis of museum specimen-derived data will follow Villablanca et. al. (1998), using the network building methods of Templeton et. al. (1992), and Templeton and Sing (1993). Confidence will be evaluated using bootstrap analysis (Hillis and Bull 1993) on parsimony and maximum likelihood phylogenies of alleles as implemented in the PAUP computer package (Swofford 2000), and constrained to reflect the results of Templeton's network analysis.

Microsatellite DNA Analysis

Microsatellite, or simple sequence repeat (SSR) DNA consists of variable numbers of very short (e.g., 2-6 base) tandemly repeated sequences (therefore known also as STR) that are not translated into functional gene products but are ubiquitous in eukaryotic genomes (Tautz et al. 1986). The length of such tandemly repeated sequences varies considerably, even among individuals, providing a rich source of polymorphisms that can be assayed using PCR (Tautz 1989). Microsatellite marker loci are codominant (like most, more familiar allozyme loci), have been demonstrated to be informative at the population level and above (Morin et al. 1992, Bruford and Wayne 1993, Potapov and Ryskov 1993, Buchanan et al. 1994, Roy et al.,1994, Meyer et al. 1995, Paetkau et al. 1995) as well as at individual and family levels (Estoup et al. 1994, Marklund et al. 1994, Zietkiewicz et al. 1994, Bancroft et al. 1995), and are obtainable from very limited amounts of dried, frozen or preserved tissue (Roy et al. 1994) and from hair (Morin et al. 1992). Microsatellite markers have been used to assign parentage (Marklund et al. 1994, Morin et al. 1994, Ellegren et al. 1995, Primmer et al. 1995), and to "fingerprint" individuals (Zietkiewicz et al. 1994), as well as to quantify evolutionary relationship, differentiation, and hybridization (Buchanan et al. 1994, Roy et al. 1994, Myer et al. 1995)

Only a few years ago, the cost and technical difficulty of identifying microsatellite loci with informative variation in a particular species and of developing primers and optimizing reaction conditions for assay of those loci, impeded their use in conservation and restoration work. Microsatellite regions in the species (or genus) to be studied first have to be identified by cloning 250-500 bp size fragments in a bacterial vector and probing with radiolabelled simple-sequence polymers (Rassmann et al. 1991). Sequencing of selected positive clones then allows PCR primers to be designed that will bracket those microsatellite regions having lengths appropriate to both PCR amplification and electrophoretic resolution of variants. It has been apparent for nearly a decade that performing these steps makes the approach suitable for large population surveys (Bruford and Wayne 1993), but impediments of time, technical expertise and expense are now substantially reduced. Today, library development, characterization, and screening of variable marker loci is a service available from specialized labs at substantially lower cost and with much shorter turn-around than has been experienced in academic laboratories.

We will outsource microsatellite library development and candidate locus identification to a commercial lab, Genetic Identification Services, Inc. (Topanga Canyon, CA). This service will return libraries enriched for microsatellite libraries, will identify at least 10 loci polymorphic in the source material we provide, and will deliver sequence information for both variable and adjacent regions which can be used to design primers for screening and population survey assays of these loci.

As codominant markers, microsatellite alleles can be analyzed with a number of standard approaches familiar to those acquainted with allozyme studies. More recent theoretical and statistical approaches have results in methods for detection and quantification of population subdivision designed specifically for microsatellite data (Excoffier and Smouse 1994, Slatkin 1995, Nielsen and Slatkin 2000). The microsatellite data obtained from Petaluma Marsh will be analyzed for both population subdivision (Nielsen 1997) and gene flow (Slatkin 1993) parameters. A procedures by Davies et al. (1999) may allow the origins of recently founded populations to be determined from the microsatellite data this study will generate.

Excellent sources for references to the most recent work in relevant areas of data analysis and for access to computer programs that will expedite analysis of molecular data are readily available on the web. Since issues and methods continually evolve in this area, further advice will be sought, if needed, from those working on these topics as the study proceeds.

Optimization of PCR-based Methods

Molecular genetics work with species (and sometimes even populations) and primers not previously studied in combination often requires optimization of PCR methods to obtain markers that can be fractionated and scored reliably. One round of PCR optimization will occur in conjunction with primer screening (template quantity and reaction components indicated by the literature to affect amplification). If informative markers are indicated for particular primers but are not well or consistently expressed, a second round of optimization may be useful before beginning population survey runs.

Fractionation and Result Recording

PCR products will be fractionated using either slab gels, capillary electrophoresis (CE), or fragment sizing methods for D-HPLC. In year 1, marker screening, methods optimization, and mtDNA population survey work will rely primarily on agarose slab gel techniques. MtDNA sequences will be asssed for size and yield on agarose gels stained with ethidium bromide and digitally photographed on a transilluminator. Microsatellite primer screening may be conducted using Metaphor or similar products for agarose gel equipment. Resolution of microsatellite allele at 10 or more loci will be achieved by fractionation on polyacrylamide gels stained with silver stain and either photographed with a digital camera (Kodak 120) on a transilluminator or read using an FMBIO II system (Miraibio, Inc./Hitachi Genetics, Alameda, CA). CE and D-HPLC equipment that may be used in years 2 and 3 to generate equivalent data via fluorescence event recording.

Data Handling and Analysis

Data generated by slab gel analysis will be obtained via digital imagery of transilluminated gels. The program Phoretix 1D provides a full suite of tools for reading, editing and verifying marker data. Similar capabilities are available in software associated with CE and D-HPLC equipment, which may be used in years 2 and 3. Data converted to marker identity and presence/absence by

one or more of these methods will then subject to analysis by standard methods used in population genetic and phylogeographic analyses.

References Cited in Appendix A

Bancroft, D.R., J.M. Pemberton, and P. King. 1995. Extensive protein and microsatellite variability in an isolated, cyclic ungulate population. Heredity 74:326-336.

Buchanan, F.C., L.J. Adams, R.P. Littlejohn, J.F. Maddox, and A.M. Crawford. 1994. Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics, 22:397-403.

Bruford, M.W., and R.K. Wayne 1993. Microsatellites and their application to population genetic studies. Current Opinion in Genetics and Development. 3:937-943.

Chenoweth, S.F., J.M. Hughes, C.P. Keenan and L. Shane. 1998. Concordance between disperssal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer* (Australian barramundi). Herredity 80:1897-197.

Davies N, G.K. Roderick, F.X.Villablanca, and S.R. Palumbi. 1999. Determining the sources of individuals in recently founded populations: multilocus genotyping in non-equilibrium genetics. Trends in Ecology and Evolution 14:17-21

Edwards, S.K. 1993. Mitochondrial gene genealogy and gene flow among island and mainland populations of a sedentary songbird, the Grey-crowned Babbler (*Pomatotomus temporalis*). Evolution 47:1118-1137.

Ellegren, H., J.T. Lifeld, T. Slagsvold and C.R. Primmer. 1995. Handicapped males and extrapair paternity in pied flycatchers: A study using microsatellite markers. Molecular Ecology 4:731-738.

Estoup, A., M. Solignac, and J.-M. Cornuet. 1994. Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. Proceedings of the Royal Society London, B. 258:1-7.

Excoffier L and Smouse PE. 1994. Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: Molecular variance parsimony. Genetics 136:343-359.

Garza, J. C. and D. S. Woodruff. 1992. A phylogentic study of the gibbons (*Hylobates*) using DNA obtained noninvasively from hair. Molecular Phylogenetics and Evolution. 1(3):202-210.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca, and A.C. Wilson. Dynamics of Mitochondrial DNA Evolution in Animals: Amplification and Sequencing With Conserved Primers. *Proc. Natl. Acad. Sci*. USA. 86:6196-6200.

Hillis, D.M. and J.J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology. 42:182-192.

Markland, S., H. Ellegren, S. Ericksson, K. Sandbert, and L. Andersson. 1994. Parentage testing and linkage analysis in the horse using a set of highly polymorphic microsatellites. Animal Genetics 25:19-23.

Morin, P.A. and D.S. Woodruff. 1996. Noninvasive genotyping for vertebrate conervation. In: Smith, T.B. and R.K. Wayne (eds). Molecular Genetic Approaches in Conservation, Oxford University Press.

Morin, P.A., J.J. Moore, and D.S. Woodruff. 1992. Identification of chimpanzee subspecies with DNA from hair and allele-specific probes. Proceedings of the Royal Society of London 249:293-297.

Morin, P.A., J. Wallis, J.J. Moore, and D.S. Woodroff. 1994. Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. Molcular Ecology 3(5):469-478.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: A critical review. Molecular Ecology 3(4):401-412.

Meyer, E, P. Wiegand, S.P. Rand, D. Kuhlmann and others. 1995. Microsatellite polymorphisms reveal phylogenetic relationships in primates. Journal of Molecular Evolution 41(1):10-14.

Nielsen, R. 1997. A Maximum Likelihood Approach to Population Samples of Microsatellite alleles. Genetics. 146: 711-716.

Nielsen, R. and M. Slatkin. 2000. Analysis of Population Subdivision using Di-Allelic Models. Evolution 54: 44-50

Palumbi, S.R. 1996. PCR and molecular systematics. In: Smith, T.B. and R.K. Wayne (eds). Molecular Genetic Approaches in Conservation, Oxford University Press.

Palumbi, S.R., and C.S. Baker. 1996. Contrasting population structure from nucelar intron sequences and mtDNA of humpback whales. Molecular Biology & Evolution 11(3):426-435.

Paetkau, D. W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347-354.

Petri, B., S. Paabo, A. Von Haeseler, and D. Tautz. 1997. Paternity assessment and population subdivision in a natural population of the larger mouse-eared bat *Myotis myotis*. Molecular Ecology 6:235-242.

Potapov, S.G., and A.P. Ryskov. 1993. [Analysis of variability of DNA repeated sequences in rodent genomes at the taxonomic level.] Genetika 29(5):869-872.

Primmer, C.R., A.P. Moller, and H. Ellegren. 1995. Resolving genetic relationships with microsatellite markers: A parentage testing system for the swallow *Hirundo rustica*. Molecular Ecology 4:493-498.

Rassmann, K., C. Schlotterer, and D. Tautz. 1991. Isolation of simple-sequence loci for use in polymerase chain reaction-based DNA fingerprinting. Electrophoresis 12:113-118.

Roy, M.S., D.J. Girman, A.C. Taylor, and R.K. Wayne. 1994. The use of museum specimens to reconstruct the genetic variability and relationships of extinct populations. Experientia 50(6):551-557.

Slatkin, M. 1995. A measure of population subdivision based on microsatellites allele frequencies. Genetics 139(1):457-462.

Swofford, D.L. 2000. PAUP* software. Washington, Smithsonian Institution.

Tautz, D. 1989. Hypervariability of simple sequences as a source for polymorphic DNA markers. Nucleic Acids Research 17:6463-6571.

Tautz, D., M. Trick, and G.A. Dover. 1986. Cryptic simplicity in DNA is a major source of genetic variation. Nature. 322:652-656.

Templeton AR, K.A. Crandall, and C.F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. cladogram estimation. *Genetics,* 132, 619-633.

Templeton, A.R., and C.F. Sing. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics,* 134, 659-669.

Thomas, W.K., S. Paabo, F.X. Villablanca, and A.C. Wilson. 1990. Spatial and Temporal Continuity of Kangaroo Rat Populations Shown by Sequencing Mitochondrial DNA from Museum Specimens. *Journal of Molecular Evolution*. 31:101-112

Villablanca, F.X., G.K. Roderick, and S.P. Palumbi. 1989. Invasion Genetics of the Mediterranean Fruit Fly: Variation in Multiple Nuclear Introns. *Molecular. Ecology*. 7:351-365.

Villablanca, F.X. 1993. Evolutionary Analysis: Spatial and Temporal Aspects of Populations Revealed by Mt. DNA. *In* Ancient DNA. B. Heermann and S. Hummel (Ed.). Springer-Verlag, N.Y. pp. 31-58.

von Beroldingen, C. H., R., G. Higuchi, G. F. Sensabaugh, and H. A. Erlich. 1987. Analysis of enzymatically amplified HLA-DQalpha DNA from single human hairs. Amer. J. of Human Genetics 41:725.

Zietkiewicz, E., A. Rafalski, and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20(2):176-183.